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Application for Research Grant
(Use extra pages as needed)

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4. Short title of study:
Interaction of chemical carcinogens and avian RNA tumor viruses

5. Proposed starting date: July 1, 1974

6. Estimated time to complete: June 30, 1976

7. Brief description of specific research aims:

1. To determine whether a chemical carcinogen can activate sarcoma genes in cells by identifying and characterizing virus particles released from methylcholanthrene-transformed quail tumor cell lines.

2. To determine whether sarcoma virus could derive sarcoma-specific genes ("oncogenes") from cells by studying changes of the virus genome when leukosis virus is serially passaged in methylcholanthrene-transformed cells.
   (a) Polyacrylamide gel electrophoresis will be used to test whether the leukosis viral RNA increases in size.
   (b) By oligonucleotide fingerprinting technique, sarcoma-specific gene sequences acquired during the course of passage in chemically transformed cells will be looked for.
   (c) Leukosis virus passaged in chemically transformed cells will be tested for focus forming activity.

3. To determine whether the transformation-specific glycoproteins and glycoprotides of avian sarcoma virus are coded for by the sarcoma virus or result from the transformed state of the cells, by comparing glycoproteins and glycopeptides of sarcoma and leukosis viruses released from chemically transformed and control cells.

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8. Brief statement of working hypothesis:

2. There is some circumstantial evidence suggesting that chemical carcinogen, such as hydrocarbon compounds, and RNA tumor virus can act synergistically to cause cellular transformation. In some in vitro studies, the exposure of tissue culture cells to both the hydrocarbon carcinogen and murine leukosis virus transformed the cells while the exposure to either one alone did not (1, 2). The most likely explanation is that the chemical carcinogens alter or activate some cellular genes, or so-called onc[illegible]nes" (3), which then act in concert with the viral genes in the leukemia virus. We have no knowledge as to how the cellular oncogene and viral genes interact.

It has been postulated that RNA tumor viruses can transduce cellular information, for instance, oncogenic genetic material (4). The phylogenetic histories of several strains of murine sarcoma viruses suggest that this is a real possibility: Kirsten sarcoma virus was isolated after prolonged passage of Kirsten murine erythroblastosis virus in rats (5), and Moloney sarcoma virus was isolated by passage of Moloney murine leukemia virus in mice (6). Furthermore, it has been shown that Kirsten sarcoma virus contained some transformation-specific genes of the rat cell lines (7). Thus it is likely that the sarcoma viruses arise by recombination between cellular transformation genes and the leukemia viral genome. However, no experimental system has been established which allows for the direct monitoring of this hypothetical recombination between virus and cell.

I propose to study the interaction between cellular transformation genes and RNA viral genomes by studying the evolution of leukosis virus during serial passage in methylcholanthrene-transformed cells. It is believed that sarcoma virus or the virus with sarcomagenic characteristic might be recovered from such cultures. The rationale behind this approach is that the chemical carcinogens might activate certain cellular transforming genes which can then be incorporated into superinfecting leukosis virus. I would concentrate on studying focus-forming activity and the structure of the viral RNA and envelope of the virus released from chemically transformed cells, since the RNA and envelope glycoproteins of sarcoma virus have been found to be distinct from those of leukosis virus (8, 9, 10).

9. Details of experimental design and procedures (append extra pages as necessary)

The basic design of this project is to infect methylcholanthrene-transformed quail cell lines with several strains of avian leukosis viruses. The viruses released from such cell lines will be studied with regard to their infectivity, transforming capability, and their structure, especially their RNA and glycoproteins. Serial passages of leukosis viruses in these cell lines will be followed. Changes in the viral characteristics monitored, particularly oncogenic properties, will be indicative of an effect of chemically transformed quail cells on the virus. These changes will be carefully investigated to reveal a possible causal relationship with cell transformation by chemical carcinogens. Parallel passages in [illegible]rmal quail fibroblasts will serve as controls.

BACKGROUND

A. Differences between avian sarcoma viruses and avian leukosis viruses.

Avian RNA tumor viruses can be roughly divided into sarcoma viruses and leukosis viruses according to their pathogenicity. Generally, sarcoma viruses transform chicken embryo fibroblasts when grown in tissue culture, while leukosis viruses do not (8). Since the transformed cells cause neoplasms upon injection into susceptible animals and also possess several other properties characteristic of tumor cells in vivo (11), sarcoma viruses are generally regarded as possessing oncogenic capability and sarcoma virus-transformed cells are used as a model for studying oncogenesis in vitro.

The isolation from avian sarcoma viruses of conditional mutants which affect only their transforming ability (12, 13, 14, 15, 16) suggests that the avian sarcoma viruses carry genes which are responsible for neoplastic transformation. The absence of such sarcoma-inducing genes from avian leukosis viruses is reflected not only in their inability to transform chicken embryo fibroblasts in vitro but also in two structural aspects of viruses : RNA and glycoproteins.

a. RNA: The virion RNA of avian RNA tumor viruses contains 1 molecule of 70S single stranded RNA plus several molecules of 4-10S RNA. THe 60-70S RNA is considered to be the viral genome and has a complex structure (17). It consists of probably three to four pieces of 30-40S subunits (18, 19) and some low molecular weight RNA (20, 21, 22). The latter may represent cellular RNA but may also include specific linkers for the 30-40S pieces as well as a primer RNA for reverse transcription (20, 23). The 30-40S RNA of avian tumor viruses can be resolved electrophoretically into 2 [illegible] classes, a larger one a and a smaller one b (8). Cloned nondefective avian sarcoma viruses contain only class a RNA; whereas leukosis viruses consist solely of class b RNA (24, 10). Transformation defective viruses derived from avian sarcoma viruses and biologically identical to leukosis

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viruses also contain only class b RNA (8). It was therefore suggested that class a RNA carried genetic information which is specific for the formation of sarcomas in the animal and for foci in fibroblast culture (8).

Furthermore, I have shown with Sanger's two-dimensional fingerprinting (electrophoresis at pH 3.5 and homochromatography) that all sequences demonstrable in class b RNA are also present in class a RNA. In addition, class a RNA appears to contain sarcoma-specific sequences which are not present in class b (10). Thus, sarcoma virus RNA can be distinguished from leukosis virus RNA by the following two ways:

(1) Electrophoresis in polyacrylamide gels reveals that sarcoma virus RNA moves slower than leukosis virus RNA.

(2) Two-dimensional fingerprinting reveals that sarcoma virus RNA contains sarcoma specific sequences which are not present in leukosis virus RNA.

These two methods will be used in this project to detect any change in genetic information in the genomes of avian leukosis viruses and to detect viruses with sarcomogenic capability.

b. Envelope glycoproteins: The avian RNA tumor virus contains an envelope which determines the host range, interference pattern and type-specific antigenicity of the virus (25). The viral envelope is composed of lipids which are mostly derived from the host membranes (26),and glycoproteins, which are coded for by the viruses (27, 9). The glycoproteins are responsible for most of the envelope properties and can be resolved into two components by polyacrylamide gel electrophoresis or by chromatography (27, 28, 29).

The size of the viral glycoprotins are correlated with the transformed state of the cells, in which the virus is grown(9). The virus released from transformed cells contain bigger glycoproteins than the virus released from nontransformed cells. Furthermore, the difference in the size of the glycoproteins is found to be primarily due to the glycopeptide moieties, obtained by exhaustive digestion of glycoproteins with pronase. The glycopeptides of viruses released from transformed cells are bigger, by approximately 1200 daltons, than those of viruses released from nontransformed cells (9). However it is not known whether the increase in the size of glycopeptides is the result of the transformed state of the host cells or the result of specific viral gene products, e.g., some glycosyl transferase, of the sarcoma virus. The methylcholanthrene-transformed quail cell would provide a system for the study of this question since the transformed state of this cell line is not caused by the transformation genes of sarcoma viruses. Thus the effects of the host cells and of the viral oncogenes can be separated.

B. Oncogene and Protovirus hypotheses

Based on some sero-epidemiological and cell culture evidence, Huebner and Todaro suggested (3, 30) that the genomes of RNA tumor viruses, "virogenes", might be part of the cellular genome and are present in all vertebrate cells, albeit in a repressed form. The occurence of cancer then might be due to the derepression of these endogenous viral genes (3, 30). Furthermore, these endogenous viral genes were considered to be the single unifying cause of cancer, as other types of carcinogens, for instance, chemical carcinogens, irradiation, or DNA tumor viruses might act through the activation of the endogenous RNA tumor viruses (3,30).

Indeed the presence of complete viral genomes in most of the normal chicken or mouse cells is indicated by nucleic acid hybridization (31, 32, 33) and by the presence of gs antigen (34) and by the chemical and physical induction of complete viruses from nearly every kind of chicken, mouse and rat cell (35, 36, 37). Therefore, it is quite likely that the viral genes are part of the cellular genes and are transmitted for generations without overt production of

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the endogenous viruses. However, the endogenous viruses induced thus far all belong to leukosis viruses which lack the transforming gene for fibroblasts. Thus, the presence of oncogene inside the cells which is responsible for the oncogenic transformation of the cells has yet to be proved.

On the other hand, Temin (4) hypothesized that RNA→DNA→RNA synthesis is a normal physiological process, and, in a rare event, might lead to the formation of genetic information for cellular transformation and viral production. The transduction of this oncogenic information by RNA tumor viurs might lead to the development of transforming sarcoma viruses.

Although these two hypotheses differ in the mechanism of the production of oncogenes and virogenes, they do agree in that the genetic information responsible for the transforming ability of the RNA tumor viruses is derived from the host cells. However, no successful attempts have been made to demonstrate such "oncogenes" inside the cells.

The following proposed project will attempt to detect such oncogenes incorporated into leukosis viruses while they are passaged in the chemically transformed cells. Since RNA tumor viruses replicate through a DNA intermediate, which is integrated into cellular chromosome (38), the recombination between viral genome and cellular transformation genes, activated by chemical carcinogens, is quite likely.

The electrophoretic analysis of RNA size and the two-dimensional fingerprinting of RNA sequences provide the sensitive techniques for the detection of any such recombination products. Even the virus which does not yet acquire transforming activity but already incorporated part of the transformation genes will be detected by these methods.

DETAILED RESEARCH PLAN

The following general methods for virus propagation will be used:

A. Cells: The cells to be used are the established quail cell lines derived from quail tumors caused by methylcholanthrene. Young adult Japanese quails (Coturnix coturnix japonica) were injected with methylcholanthrene intramuscularly, and tumors developed within one to two months. The tumors have been established in tissue culture as continuous lines by Dr. C. Moscovici and eight different tumor lines have been received from Dr. C. Moscovici and are now maintained in this laboratory.

For focus assays, the C/O and C/E fibroblasts derived from 11-day-old chicken embryos will be used.

B. Viruses: The quail cells are susceptible to avian tumor viruses of subgroups A, E and F and are resistant to the viruses of other subgroups (39, 40, 41). The leukosis viruses or transformation-defective viruses belonging to these subgroups, e.g., RAV-1 (Rous-associated virus), RAV-3, RAV-5, td PR RSV-A (a transformation-defective derivative of Prague strain Rous sarcoma virus), lymphoid leukosis virus RPL-12, RAV-60, or RAV-61 will be used to infect the quail tumor cells.

The quail tumor cells will be infected with various leukosis viruses at multiplicity of infection about 1. Daily medium changes are required. At day 4, the cells will be trypsinized and reseeded. This transfer increases the titer of viruses released into the medium. At day 7 or 8, the cells are confluent and will be labeled with radioactive precursors. The viruses will be purified from the medium by ammonium sulfate precipitation, sedimentation to a

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65%-20% sucrose interface, and finally equilibrium sedimentation. The virus has a density of 1.16-1.18 gm% (18). The RNA will be extracted with sodium dodecyl sulfate and phenol (18). The 70S RNA will be separated from 4S RNA by velocity sedimentation in sucrose gradients (18).

I. Do methylcholanthrene-transformed quail cells release virus particles?

Methylcholanthrene can transform mouse, rat, and hamster cells in vitro under a variety of conditions (42, 43). These chemically transformed cells have not been observed to produce any infectious tumor viruses (44). On the other hand, chemically induced tumors have been found in several cases to release tumor viruses (51, 52, 53). Furthermore, methylcholanthrene has also been used to induce the expression of the genome of endogenous RNA tumor viruses from normal chicken cells (35). The extent of induction ranges from production of the infectious virus particles to conversion of chicken cells from gs (-) to gs (+). It is therefore necessary to study the physiological states of the methylcholanthrene-transformed quail tumor cells which I plan to use in this project. The main questions are:

(1) Are the quail tumor cell lines gs (+), i.e., contain the group-specific antigen of avian tumor viruses?

(2) Do they produce infectious viruses?

(3) If no, do they produce any physical particles resembling RNA tumor viruses?

The gs test has been established as a routine test for our primary chicken embryo fibroblast cultures in this laboratory. The method is as described by Vogt and Friis (54). It is a complement-fixation test for the detection of group-specific antigens in the cells. If the quail tumor cells turn out to be positive for gs antigen of avian tumor virus, while the normal quail cells from the same flock are negative, it would show that the normal quail cells contain the genome of avian viruses and the chemical carcinogen induces the partial expression of these gene functions.

The detection of focus-forming viruses released into the culture media will be done by the routine focus assay which has been developed by Rubin (45) and modified by Vogt (46). The focus assay will be performed on C/O chicken cells, which are susceptible to all the known strains of avian tumor viruses.

The detection of noninfectious viral particles will be done by radioactive labeling of cultured quail tumor cells. ^{3}H-uridine or ^{3}H-amino acids will be added to the culture medium, and the latter will be purified to detect any particles which have a density around 1.16 gm/ml and 1.18 gm/ml, the density of the RNA tumor viruses. These virus particles might represent expression of endogenous tumor viral genes.

Preliminary data suggest that, in one quail tumor cell line, a transforming virus is produced (Moscovici and Vogt, personal communication). In another cell line, release of noninfectious virus particles has been observed. These viruses will be studied in more details. Their RNA will be labeled with ^{3}H-uridine or ^{32}P, and analyzed by polyacrylamide gel electrophoresis, to see whether they contain class a or class b RNA, or RNA of even smaller size. It will also be digested with RNase T_1 and analyzed by two-dimensional electrophoresis and homochromatography to see whether it lacks any gene sequences. The

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protein structure of these particles will be studied by electrophoresis on 10% polyacrylamide gels to see whether they lack any protein components. This again will suggest that the genome of RNA tumor virus is present in the quail cells, and that the chemical carcinogen, methylcholanthrene, acts as an inducer for these viruses.

The quail tumor cell lines which produce infectious or noninfectious virus particles will not be used for the following study, since the endogenous virus is difficult to be distinguished from the superinfecting virus.

II. Serial passage of avian luekosis virus in the methylcholanthrene-transformed cell lines of the Japanese quail.

This experiment is designed to detect any change in the viral genome during serial passage of an RNA leukosis virus in methylcholanthrene-transformed cell lines. Transformed cell lines which do not produce any infectious or noninfectious virus particles will be infected with several strains of avian luekosis viruses. Most of the lines are gs (-), but gs (+) lines will also be used, if available. They will be studied separately. Since the purpose of this experiment is to detect acquisition of sarcomagenic potential by leukosis virus during the course of infection, only leukosis viruses will be used for the initial infection.

Quail cells have been shown to be susceptible to avian tumor viruses of subgroups A, E and F (39, 40, 41). They are relatively resistant to leukosis viruses of subgroups B, C and D (39). Subgroups E and F include endogenous viruses and induced leukosis viruses of chicken and ringnecked phaesant (40, 41). No natural sarcoma viruses have been found to fall into these two subgroups. It is not known whether this is due to the lack of recombination ability with host sarcoma genes in the endogenous viruses. Because of this peculiar feature of subgroups E and F, leukosis viruses and transformation-defective derivatives of sarcoma viruses belonging to subgroup A will be used first. Some examples of viruses to be used are Rous associated viruses, RAV-1, RAV-3, RAV-5, lymphoid leukosis virus, RPL-12 and the transformation defective derivative of Prague strain of RSV (td PR-RSV-A). The cells will be infected at a multiplicity of infection about 1. At day 7 or thereafter, when the cells are confluent, the culture medium will be collected and used for the virus stock to infect the new cells. These new cells will be regarded as the second passage virus. This virus will be used to infect the new cells ("third passage cells") again.

This cycle will be carried on. At the end of each passage, the cells will be labeled with ^{3}H-uridine or ^{32}P. The virus will be purified from the culture medium. The viral RNA will be analyzed in the following ways:

(1) polyacrylamide gel electrophoresis: All the leukosis viruses which will be used to infect the quail cell lines contain class b RNA (24). Presumably this RNA lacks some sarcoma gene sequences which are present in class a RNA of sarcoma virus (10). If the leukosis viruses acquire some sarcomagenic sequences from the host tumor cells during the course of transmission in methylcholanthrene-transformed cells, it will be expected that the size of RNA of the leukosis viruses will be bigger than class b RNA. The mechanism of integration of sarcomagenic information into leukosis viral genome might be through genetic recombination, which has been demonstrated to occur between endogenous virus and the superinfecting sarcoma virus (47). The genetic recombination between avian tumor viruses which results in the increase of viral genome size has also been demonstrated (Duesberg, personal communication). This recombination could be

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a stepwise process. The leukosis virus may pick up part of the transformation genes first. As the virus is serially passaged through the methylcholanthrene-transformed cell lines, the leukosis virus might acquire more sarcomogenic genes, and eventually their RNA reaches the size of class a RNA.

The superinfected tumor cell lines will be labeled with ^{32}P. The culture medium will be collected at three-hour intervals to minimize the degradation of viral RNA in the culture medium. The virus will be purified from the pooled culture medium. The 60-70S RNA of the virus will be mixed with the ^{3}H-uridine-labeled 60-70S RNA of the leukosis virus propagated in the normal quail embryo fibroblasts as a control. The RNA mixture will be heated at 100° C for 45 seconds (18) and the resulting 30-40S RNA will be electrophoresed in the 2% polyacrylamide gels. Any changes in the size of the viral RNA of leukosis virus propagated in the methylcholanthrene-transformed cell lines can be detected.

If the viral RNA increases in size, it will suggest that some cellular genes have been incorporated into viral genome. However, it is also likely that this is due to the amplification of part of the viral genome. To determine that cellular genes, particularly, sarcoma-specific genes, have been incorporated into the virus, oligonucleotide fingerprinting technique and focus assay of the virus will also be used.

(2) oligonucleotide fingerprinting: Sanger's two-dimensional fingerprinting method for RNA gave characteristic fingerprints for every strain of RNA tumor viruses (10; Lai, unpulblished observation). This method involves digesting the RNA with RNase T_1 and then separating the resulting RNase T_1-resistant oligonucleotides of the RNA by electrophoresis at pH 3.5 in the first dimension and homochromatography in the second dimension. About 20-25 larger oligonucleotides (10-50 nucleotides long), which account for about 2-3% of the total RNA, are resolvable, and their distribution patterns are the basis for characterizing the RNA. The rest of the RNA is degraded into oligonucleotides too small to be significant. I have used this method to compare the RNA of sarcoma viruses with that of leukosis viruses. It was found that all the genetic sequences in leukosis viruses are present in sarcoma viruses. Moreover, sarcoma viruses contain a few additional oligonucleotide sequences which are sarcoma-specific (10). These sarcoma-specific sequences could be used for identifying sarcoma genome.

Thus the ^{32}P-labeled 30-40S RNA of the leukosis viruses released from quail tumor cell lines and prepared according to the method described in the last section will be analyzed by the two-dimensional fingerprinting. If they incorporated the sarcoma-specific gene sequences from the host cells, the sarcoma-specific sequences identified previously (10) should appear in the fingerprints of the leukosis viruses released from quail tumor cell lines. However, since the RNA sequences identifiable by this method constitute only 2-3% of the total RNA sequences, this method of identifying RNase T_1-resistant oligonucleotides should be less sensitive than the polyacrylamide gel electrophoresis of RNA. Failure to find the sarcoma-specific oligonucleotides does not rule out the possibility that part of the transformation genes is already integrated into the viral genome. The ultimate proof that the virus has incorporated the sarcoma-specific genes in the cells should come from the demonstration that the virus released from chemically transformed cells can produce foci on chicken embryo fibroblasts.

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(3) focus assay of viruses released from quail cell lines: The 30-40S RNA of sarcoma viruses is larger than that of leukosis viruses by about 6 x 10^5 daltons in molecular weight, which can code for one to two protein molecules (48). Furthermore, there are two to four complementation groups for the transformation function (13, 49), suggesting that there are 2 to 4 genes required for cellular transformation. For a sarcoma virus to possess transforming ability, it might be necessary to incorporate all the 2-4 transforming genes into its genome. However, the acquisition of sarcoma genes from the chemically transformed cells by superinfecting leukosis viruses might be gradual and stepwise, picking up one gene at a time. The incorporation of the complete sarcoma genes and, in turn, the emergence of sarcoma virus, might be a very rare event. From this point of view, the focus-forming activity of the viruses released from chemically transformed cells might not be a very sensitive technique. On the other hand, it is likely that replication-defective sarcoma virus might appear first as a result of recombination between cellular sarcoma genes and part of viral genes. The RNA of this virus could not be distinguished by size from class b RNA of the superinfecting leukosis virus. One example of this class of replication-defective sarcoma virus is Bryan high titer strain of Rous sarcoma virus, which produces foci on chicken embryo fibroblasts, but contains class b RNA (55, 56). This virus could not be detected by polyacrylamide gel electrophoresis of the RNA of the viruses released from chemically transformed cells. However, it could be detected by focus assays, since the released viruses would contain a mixture of leukosis virus and pseudotype virus thus complementing the replication-defective sarcoma virus (57).

Focus assays will be done on C/O chicken embryo fibroblasts according to the method of Vogt (46). The culture medium in each passage will be checked for focus-forming units. If focus-forming virus can be detected in the culture medium, it would suggest that complete sarcoma genes have been incorporated into the viral genome.

Some problems might be anticipated in harvesting virus from the culture medium of quail tumor cell lines. Firstly, Friis (50) observed that the virus replication of Rous sarcoma virus, particularly with B77 of subgroup C and to a lesser degree, Prague strain of subgroup A, in quail cells is restricted. Very low titers of viruses are released into the medium. However, our preliminary data using RSV (RAV-3) (a pseudotype virus of subgroup A) to infect quail tumor cell lines suggested that subgroup A viruses are released in sufficient amounts for these experiments. Secondly, it has been observed (Lai, unpublished observation) that it is difficult to harvest the radioactively labeled leukosis viruses of subgroup A from culture medium. There is no such difficulty with sarcoma viruses of subgroup A, suggesting that the transformed state, perhaps the membrane properties of the host cells determine whether the viruses are released into the medium or not. The preliminary data suggested that we might have difficulty in harvesting leukosis virus from chemically transformed cells too. However, this difficulty can be partially overcome by sonication of culture medium prior to virus purification, suggesting that the virus particles of subgroups A might clump in the culture medium and that the difficulty in getting virus from culture medium might be an artifact. This phenomenon will be further investigated by studying the effects of polybrene or trypsin on the release of the virus. These difficulties are not encountered with subgroup E and F viruses, which will also be used in these studies.

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III. Identification of leukosis viruses released from quail tumor cells.

If leukosis viruses containing bigger 30-40S RNA than class b RNA, or focus-forming sarcoma viruses are released from the chemically transformed quail tumor cells during the course of serial passages, further identification of viruses becomes necessary. There are two basic reasons:

(1) Methylcholanthrene is known to induce the endogenous virus (35).

(2) Infection of avian cells with some strains of avian tumor viruses can also induce endogenous viruses (40, 41).

Therefore the viruses released during the serial passages of the leukosis virus in the methylcholanthrene-transformed cells might be endogenous viruses, instead of the superinfecting viruses with incorporated host genes. Thus the viruses will be classified with regard to their host range, interference pattern and antigenicity (39). The classification techniques are established and all the virus strains are available in our laboratory. Since the superinfecting virus belongs to subgroup A and the one endogenous virus of quail cells isolated so far belongs to subgroup C (Vogt, personal communication), the two viruses can be easily distinguished. Furthermore, since every virus strain has its characteristic T_1-oligonucleotide fingerprint (Lai, unpublished observation), the virus strains can be identified by studying its oligonucleotide fingerprint using the two-dimensional fingerprinting technique as described above. This will be used for further identification of the virus released from quail tumor cells. If it is the virus derived from the superinfecting leukosis virus, e.g., RAV-1 or td PR-A, it should share the subgroup-specific oligonucleotides with the virus grown in normal quail or chicken cells.

IV. The size of the glycoproteins and glycopeptides of the virus released from the chemically transformed cells.

The ^{3}H- or ^{14}C-glucosamine-labeled glycoproteins of RAV-1 or td PR-RSV-A released from the methylcholanthrene-transformed quail tumor cells will be compared with those of RAV-1 or td PR RSV-A released from the normal quail cells respectively. The glycoproteins will be coelectrophoresed in 5% polyacrylamide gels to compare their size. The glycopeptides will be obtained by exhaustive digestion of viral glycoproteins with pronase (1 mg/ml) and then analyzed by sephadex chromatography.

The glycoproteins and glycopeptides of RAV-1 or td PR-RSV-A released from the methylcholanthrene-transformed quail cells will also be compared with those of viruses released from RSV(RAV-1) or PR RSV-A transformed quail cells.

It has been shown that the viruses released from virus-transformed cells have bigger glycoproteins than those from nontransformed cells (9). If the glycoproteins and glycopeptides of viruses released from the chemically transformed cells have similar sizes to those of transforming sarcoma viruses, e.g., RSV(RAV-1) or PR RSV-A, it will suggest that the large size of the glycoproteins and glycopeptides in the viruses released from the transformed cells is the result of the transformed state of the host cells. It will also suggest that methylcholanthrene-transformed cells have similar physiological state to virus-transformed cells. On the other hand, if their sizes are similar to those of leukosis

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viruses, i.e., RAV-1 or td PR-RSV-A, grown in normal cells, it will suggest that the large glycoproteins and glycopeptides are coded for by the sarcoma viral genome.

When the viruses with incorporated cellular genes are obtained, their glycoproteins and glycopeptides will also be studied in the same way to see whether there is any change in their sizes.

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10. Space and facilities available (when elsewhere than item 2 indicates, state location):

The space available to me includes 600 square feet of laboratory space, kitchen space for glassware washing, animal room and secretarial office space which is provided in conjunction with the Department of Microbiology office.

The Department of Microbiology has provided me with part of high-voltage electrophoresis apparatus, including one high voltage power supply and one electrophoresis tank, one Wedco incubator and one refrigerator. I also have access to one Sorvall centrifuge, one Beckman centrifuge, 4 Spinco ultracentrifuges equipped with SW 50.1, SW41, SW27 and SW25.2 rotors, 2 Packard scintillation counters, one Gilford spectrophotometer and one darkroom.

11. Additional facilities required:

1. Inverted Microscope for daily examination of tissue culture cells (requested in this application.

2. Revco Deep Freezer. Savant electrophoresis tank, Spinco SW65 rotor, Corning still, Fraction collector: requested from Public Health Service and American Cancer Society. Still pending.

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12. Biographical sketches of investigator(s) and other professional personnel (append):

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

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12. Biographical Data

Personal Information:

Name:	Michael Ming-Chiao Lai
Business Address:	Department of Microbiology University of Southern California School of Medicine 2025 Zonal Avenue Los Angeles, California 90033
Business Phone:	A.C 213· 225-1511, Ext. 308
Date of Birth:	REDACTED
Place of Birth:	REDACTED
Citizenship:	
Sex:	Male
Marital Status:	REDACTED

Education:

REDACTED

National Taiwan University College of Medicine, Taipei
M. D. (Bachelor of Medicine)
Intern, National Taiwan University Hospital
Internist Officer, Chinese Marine Corps
Department of Molecular Biology and Virus Laboratory
University of California, Berkeley
Ph. D. in Molecular Biology

Professional Background:

1973 to present	Assistant Professor, Department of Microbiology University of Southern California School of Medicine
1973 (January-June)	Postgraduate Research Molecular Biologist Department of Molecular Biology, University of California, Berkeley
1969-1972	Research and Teaching Assistant Department of Molecular Biology and Virus Laboratory University of California, Berkeley
1968-1969	Internist Officer Chinese Marine Corps
1968	Passed doctor's qualification Educational Council for Foreign Medical Graduates
1967-1968	Intern National Taiwan University Hospital
1961-1967	Medical Student National Taiwan University College of Medicine
1965, 1964	Summer Research Student Department of Biochemistry, National Taiwan University

Honors:

M. D. - Summa cum laude (1968)

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13. Publications:

Lai, M. C. (1968) A study of sex chromatin in normal Chinese newborns. M. D. thesis. National Taiwan University College of Medicine.

Lai, M. M. C. and P. H. Duesberg (1972). Adenylic acid-rich sequences in RNAs of Rous sarcoma virus and Rauscher mouse leukemia virus. Nature 235: 383-386.

Lai, M. M. C. and P. H. Duesberg (1972). Differences between the envelope glycoproteins and glycopeptides of avian tumor viruses released from transformed and from nontransformed cells. Virology 50: 359-372.

Lai, M. M. C. (1973). On the structure of RNA tumor viruses. Ph. D. dissertation. University of California, Berkeley.

Lai, M. M. C., P. H. Duesberg, J. Horst and P. K. Vogt (1973). Avian tumor virus RNA: A comparison of three sarcoma viruses and their transformation defective derivatives by oligonucleotide fingerprinting and DNA-RNA hybridization. Proc. Nat. Acad. Sci. U. S. 70: 2266-2270.

Lai, M. M. C., J. Horst, and P. H. Duesberg (1973). The RNA of nondefective avian sarcoma viruses and corresponding transformation defective segregants: A comparison by oligonucleotide fingerprinting and RNA-DNA hybridization. Virus Research, (2nd ICN-UCLA Symposium on Molecular Biology) (Eds. F. Fox and W. Robinson), p. 345.

Duesberg, P. H., E. Canaani, M. M. C. Lai and P. K. Vogt (1972). News and reviews on avian tumor virus RNA (Abstract). IV. Lepetit Colloquium Cocoyoc, Mexico.

Duesberg, P. H., E. Canaani, K. v. d. Helm, M. M. C. Lai and P. K. Vogt (1973). News and reviews on avian tumor virus RNA. In: Possible Episomes in Eukaryotes (IV. Lepitit Colloquium) (Ed. L. G. Silvestri). In press.

Duesberg, P. H., P. K. Vogt, J. Maisel, M. M. C. Lai and E. Canaani (1973). Tracking defective tumor virus RNA. Virus Research (2nd ICN-UCLA Symposium on Molecular Biology) (Eds. F. Fox and W. Robinson), p. 327.

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Duesberg, P. H., M. M. C. Lai and J. Maisel (1973) Tumor virus RNAs and tumor virus genes. In "Modern Trends in Human Leukemia - Biological, Biochemical and Virological Aspects". (Eds. R. Neth, R. Gallo and F. Stohlman). J. F. Lehmanns Verlag München, Germany. In press.

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14. First year budget:

A. Salaries (give names or state "to be recruited") Professional (give % time of investigator(s) even if no salary requested)	% time	Amount
1. Michael M.C. LAI, Principal Investigator (74-75)	40%	----
Lai, 1/9 of annual salary - summer supplement (see Justification, 4a)	100%	REDACTED
2. Research Assistant (to be recruited)	9 months 50%	REDACTED
	3 months 100%	
Technical		
Lab Helper (to be recruited)	20 hours/week	REDACTED
	subtotal	
12% S and W F.B.		REDACTED
Sub-Total for A		REDACTED

B. Consumable supplies (by major categories)	
Radioisotopes	5100
Sera, media, chemicals	2300
Scintillation vials, and counting fluids	1600
Disposable tissue culture supplies	1100
Glassware	600
Sub-Total for B	10,700

Budget okay

C. Other expenses (itemize)	
Service contract; repair	1065
Xerox	300
Telephone	200
Publication costs	250
Sub-Total for C	1,815
Running Total of A + B + C	23,398

D. Permanent equipment (itemize)	
1. Zeiss Inverted Microscope	1250
2. Mettler top loading balance	980
3. Lakeside Carts	175
4. Refrigerator	360
5. Virtis pipette dryer	250
Sub-Total for D	3,015
E. Indirect costs (15% of A+B+C)	3,510
Total request	29,923

15. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	R	10,400	3,577	1,050	4,315	34,131
Year 3	R	11,696	4,006	----	4,771	36,530

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Justification of Budget:

Salaries: The salary of the Principal Investigator represents a summer salary as per University policy. The University of Southern California is on a nine-month salary scale. The Principal Investigator will not take vacation during the summer months. Two ninths of annual salaries were requested from Public Health Service. The salaries requested in this application represents one ninth of the nine-month salaries. During the second and third years, the laboratory helper will become full-time. Increases in salaries are based on the past fiscal average merit increases as per University policy.

Consumable supplies: Radioisotopes - most of the experiments proposed in this project involves the use of radioactive chemicals. The following figures are rough estimates of major radioisotopes to be consumed in a year on this project:

^{32}P: 1,000 mCurie	($1200)
^{3}H-uridine: 100 mCurie	(900)
^{3}H-glucosamine: 30 mCurie	(1500)
^{14}C-glucosamine: 3 mCurie	(1500)

Scintillation vials and counting supplies: Virus purification, RNA preparation by sucrose gradients, polyacrylamide gel electrophoresis, column chromatography all require counting in a liquid scintillation counter. About 800 vials per month will be used for this project.

Glassware: To avoid contamination by RNase, most of the biochemical glassware to be used will be disposable.

Plasticware, sera, media and chemicals: This project involves the use of large amounts of tissue culture.

Equipment: Inverted microscope: necessary for daily examination of tissue culture cells. Presently no microscope is available to me in the laboratory.

Mettler balance: necessary for various measurements in biochemical studies.

Carts: necessary for various purposes in the laboratory.

Refrigerator: Presently there is only one refrigerator in the laboratory. One more refrigerator is required for storing tissue culture media and biochemical materials separately.

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16. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
1) Structure and Replication of Rous Sarcoma Virus	Institutional Grant from American Cancer Society	$3,000	Aug. 15, 1973 - Apr. 30, 1974
2) Structure and Replication of Rous Sarcoma Virus	California Division of American Cancer Society (Special grant #656)	$10,000	Oct. 1, 1973 - Sept. 30, 1974

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Structure and Replication of Rous Sarcoma Virus	1) Public Health Service Dept. of Health, Education and Welfare (CA 16113-01)	$159,307	Pending (May 1, 1974 - Apr. 30, 1977)
	2) American Cancer Soc.	$185,650	Pending (July 1, 1974 - June 30, 1977)
	Only one of the above, if awarded, will be accepted.		

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Michael Ming-Chiao LAI

Signature Michael Ming-Chiao Lai Date 1-25-74

Telephone 213 (Area Code) 225-1511 (Number) x 208 (Extension)

Checks payable to

University of Southern California

Mailing address for checks

University Park

Los Angeles, Ca. 90024

Responsible officer of institution

Typed Name Z. A. Kaprielian

Title Vice Pres. Academic Administration and Research

Signature _____ Date _____

Telephone 213 (Area Code) 225-1511 (Number) x 432 (Extension)

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